

EFFECT OF TWELVE ANNUAL APPLICATIONS OF DIURON, SIMAZINE, AND TERBACIL ON A SOIL MICROBE COMMUNITY IN WEST VIRGINIA

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ABSTRACT

Diuron, simazine, and terbacil were applied annually at rates of 4 lb/A to separate plots near Kearneysville, WV from 1981 through 1995. Untreated controls and spring and fall cultivation treatments were included. Components of the soil microbial community were measured in 1993 and 1994. The number of colony forming units (CFU) of bacteria and actinomycete per g soil was greatest in cultivated plots (7.1×10^6) and least in terbacil-treated plots (1.6×10^6). The number of gram-negative bacteria and fungi were least in terbacil-treated plots (3.3×10^5 and 2.2×10^4 CFU/g, respectively) but were not significantly different from other treatments. Colony forming units in all soil components from simazine and diuron-treated plots were between those from control and terbacil-treated plots. Low CFU from terbacil-treated plots may be partly due to reduced vegetation (less than 5% vegetative cover in Fall) compared to control (more than 80% cover) and the consequent reduction of soil organic matter (32% less than control).

INTRODUCTION

Simazine (6-chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine), diuron (N'-(3,4-dichlorophenyl)-N,N-dimethylurea), and terbacil (5-chloro-3-(1,1-dimethylethyl)-6-methyl-2,4(1H,3H)-pyrimidinedione) are used as preemergence herbicides to control weeds near fruit trees. These herbicides have been available for over thirty years and in some instances have been applied repeatedly within an orchard. They do not leach readily from the soil, and do not accumulate in large quantities. But several annual applications can contribute to soil residues (3, 9, 10). Diuron and simazine are biologically degraded and serve as a resource for some soil microbe populations (2, 10). In some instances, herbicides have stimulated certain soil microflora components while inhibiting others (4, 6). Effects of long-term applications of herbicides are further complicated by soil herbicide-sorption kinetics that differ between recent and aged residues (11). In this experiment, three components of the soil microflora community were measured following twelve annual applications of diuron, simazine, and terbacil.

MATERIALS AND METHODS

Field. Plots (5 X 50 ft) were selected in a randomized block design in a 2-acre field near Kearneysville, WV. complete
Beginning

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in the Spring of 1981, simazine (Princep, 80% WP), diuron (Karmex, 80% dispersible granule), and terbacil (Sinbar, 80% WP) were applied to separate plots at rates of 4 lb ai/acre. Herbicides were applied over the top with a 4-nozzle boom equipped with Delavan LF-F tips on 20-in spacing, in a carrier volume of 58.7 gal/A. Herbicides were applied between the first weeks of May and June each year. In addition to herbicide treatments, control plots were maintained. Ailanthus altissima were dominant on control plots and were sheared annually to a 1 ft height each winter in order to slow ecological succession. A cultivation treatment was applied to separate plots, with cultivation to a depth of 20 cm in May and October each year. Average weekly soil temperatures were recorded and soil physical and chemical properties were determined. Vegetation was estimated visually in June and September as the percent ground area covered.

Field microflora components. Soil was sampled three times during 1993 and 1994: 1 week before herbicide treatment, 1 week after herbicide treatment, and 6 weeks after herbicide treatment. A 2.54-cm diameter soil probe was used to remove 5 subsamples per plot to a 20 cm depth. Preliminary work demonstrated only a small microbial presence at depths below 20 cm. The 5 subsamples per plot were pooled and each sample was quantitatively evaluated for total bacteria and actinomycetes, gram-negative bacteria, and fungi.

Soil from each plot was mixed, stones were removed, and 10 g was placed in 90 mL phosphate buffer (50mM, pH 7.0) for 60 min and shook on an orbital shaker (1,000 rpm). Serial dilutions with buffer were made with concentrations from 10^{-1} to 10^{-6} .

Bacteria and actinomycetes. Dilute nutrient agar was prepared by mixing 0.8 g Bacto Nutrient Broth with 18.0 g Bacto Agar in 1 L water and sterilized at 121 C and 15 psi for 20 min. Cycloheximide was added to the agar following sterilization to yield a 100 ug mL^{-1} concentration. One-hundred μL of two dilutions (10^{-3} and 10^{-4}) were spiral plated [Spiral Systems, Bethesda, MD] on two petri dishes per dilution. Plates were incubated at 25 C and total number of actinomycete and bacterial colony forming units (CFU) were counted [Model 500A CFU counter, Spiral Systems] after 5 days.

Gram-negative bacteria. Dilute nutrient agar was prepared as described above and crystal violet was added after sterilization to a final 2 ug mL^{-1} concentration (5). One-hundred μL of two dilutions (10^{-3} and 10^{-4}) were spiral plated on two petri dishes and CFUs were counted as described above.

Fungi. Potato dextrose agar (19.5 g in 500 mL H_2O) was sterilized and streptomycin and rose bengal were added to final concentrations of 300 and 2 ug mL^{-1} , respectively (7). One-hundred μL of the 10^{-2} and 10^{-3} dilutions were spread on two plates per dilution. Plates were then evaluated following 5 days

incubation for fungal CFUs.

Experimental Design. The field experimental design was completely random with 5 treatments and 4 replications. Analysis was completed by GLM (SAS).

RESULTS AND DISCUSSION

Weed management had little effect on soil fungi and no effect on gram-negative bacteria in the soil (Table 1). Diuron treated plots had more fungi than terbacil treated plots. Total bacteria and actinomycete were affected by weed management. Cultivated plots had twice and four-fold more bacteria CFUs than simazine and terbacil plots, respectively. Cultivated plots had greater organic matter content than terbacil treated plots (1.7 vs. 1.3%, respectively). Aeration resulting from the cultivations may also have stimulated bacteria and actinomycete growth. Bacteria and actinomycete CFUs did not differ between control and any herbicide treated plot. These herbicides, therefore, did not cause large toxic or stimulatory effects on the microbial community. Skipper and Volk (1972) also found a weak relationship between microbial population numbers and the capacity to biodegrade atrazine [2-chloro-4-(ethylamino) s-triazine]. However, qualitative changes may have occurred within the microbial community. In this experiment, individual bacteria species were not measured.

Differences in CFUs were associated with time of measurement (Table 1). Fungi CFUs declined while total bacteria CFUs increased after May. Seasonal patterns of increasing temperatures to a range from 27 to 32 C probably contributed to these trends (1). Gram-negative bacteria decreased between May and June and then increased between June and August. These differences do not correlate with herbicide application. No statistical interaction was found between weed management treatment and time of measurement. An interaction was expected if a component of the soil microbial community was stimulated or inhibited by a herbicide.

In this experiment, the only notable difference among soil microbe components due to herbicide treatment was reduced CFUs in soil from terbacil treated plots (Table 1). These plots had less than 5% vegetation coverage in contrast to all other plots that had at least 50% coverage. This lack of vegetation contributed to decreased soil organic matter (a microbial energy source) and to soil compaction that reduced aeration. It is likely that these alterations in the soil environment contributed to the reduced CFUs. In addition, we performed experiments that demonstrated that herbicide-degradation capacity of soil microbes was not different between control plots and plots which had been treated with herbicides (data not shown).

This experiment was conducted under conditions (soil, climate, and longevity of application) that are likely for peach orchards

in the Shenandoah Valley and Cumberland Plateau region of the eastern U.S. Microbial diversity was not quantitated, but clearly the total number of microbes was not reduced by diuron and simazine. There was no evidence for selection for herbicide-degrading microbes following repeated herbicide application. The results indicate that long-term use of diuron, simazine, and terbacil under the conditions in this experiment does not dramatically alter the soil microbial community..

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Table 1. Effects of past weed management practices on selected components of soil microflora.

Main effects ^a	Fungi	Microflora Components Gram-negative bacteria	Total bacteria and actinomycetes
(cfu x 10 ⁵ /g soil d.w.) ^b			
Weed Management			
Control	0.40ab ^c	5.99a	38.58abc
Cultivated	0.36ab	7.08a	70.97a
Diuron	0.43a	4.98a	58.24ab
Simazine	0.31ab	5.45a	35.65bc
Terbacil	0.22b	3.27a	15.98c
Time Measured			
May	0.52a	6.91a	28.06b
June	0.25b	3.31b	39.00ab
Aug	0.26b	5.85ab	64.59a

^a No interaction occurred between weed management practice and time of measurement. May, June, and August measurements were 1 week before, 1 week after, and 6 weeks after weed management, respectively.

^b cfu is colony forming units estimated by dilution plating.

^c Within a column and main effect, means followed by the same letter do not differ at the 0.05 level according to Duncan's Multiple Range Test.